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Research Article

PIOGLITAZONE AUGMENTS ACETYL CHOLINE INDUCED CALCIUM MEDIATED SMOOTH MUSCLE CONTRACTIONS IN CHICK ILEUM

Divya. S*, Kavimani. S, Subalakshmi. M, Saranya. A and Murali. R

Department of Pharmacology, Mother Theresa Post graduate and Research Institute of Health Sciences, Puducherry-605006, India.

ABSTRACT

Pioglitazone, a thiazolidinedione modulate the expression of insulin sensitive genes in insulin target tissues like liver and adipose tissue by activating the nuclear peroxisome proliferator activated receptor gamma (PPAR-γ) and thereby alleviate insulin resistance. As increased intracellular calcium has been shown to induce insulin secretion, the possible role of pioglitazone on pancreas and insulin release can be ascertained by its effect on calcium release. The present study was designed to study the effect of pioglitazone on Acetyl choline (Ach) induced smooth muscle contraction which is a calcium mediated activity. Pioglitazone was used at two different concentrations - 4µg/ml and 8µg/ml. Pioglitazone treatment induced significant increase in Ach induced smooth muscle contractions in chick ileum in a dose dependent manner. With 100 µg of Ach alone the concentration response curve showed a response with a height of 96mm while in the presence of pioglitazone, the response height of 115mm and 127mm was produced at 4µg/ml and 8µg/ml respectively. This effect of pioglitazone may be attributed to its effect on augmentation of cytoplasmic calcium levels.

Keywords: Thiazolidinediones, Peroxisome Proliferator Activated Receptor gamma(PPAR- **γ**).

1.0 INTRODUCTION

Thiazolidinedione's (TZDs) are newer class of drugs that were initially identified for their insulin sensitizing properties. Pioglitazone, a thiazolidinedione acts to decrease insulin resistance and enhance insulin action in target tissues. It activates the nuclear peroxisome proliferated activated receptor (PPAR-γ), a nuclear orphan receptor that is predominantly expressed in adipose tissue and to a lesser extent in muscle, liver and other tissues. The endogenous ligand for PPAR- γ receptor is postulated to be prostaglandin J_2 and it appears to work by heterodimerizing with other nuclear receptors to modulate the expression of insulin sensitive genes.(Charles R. Craig, Robert E. Stitzel, 2012).

1.1. Isolated Chick Ileum- A Brief Introduction

Chick ileum is an isolated tissue preparation used for bioassay of drugs. Experiments with this isolated chick ileum are chiefly designed to understand the receptor action of drugs *in-vitro* with an emphasis to appreciate the concepts of graded response, nature of antagonism, potentiation and dose ratios.(Kulkarni, 1999).

The chick ileum contains cholinergic muscarinic receptors, serotonergic receptors, histaminic(H_1&H_2) receptors, prostaglandin receptors (PGE,PGE_2,$PGF_{2\alpha}$),progesterone receptors, tachykinin receptors and motilin receptors(Hansen *et al*.,1989;Salolaa *et al*.,1989;Pasanen *et al*.,1997;Kitazawa *et al*.,1997;Liu and Burcher 2001; Martin *et al.,*1993; Pust *et al*.,1996).

1.2 Drugs Acting On Chick Ileum

- Histamine (H_1) produces contraction. H_2 receptors produce relaxation of the ileum(Chand et al.,1978).
- Metiamide (H_2 receptor antagonist) blocked histamine (H_2) induced relaxation and potentiate contractile response to histamine(H_1)(Pasanen *et al.,*1997).
- Tachykinin produce contraction of the chick ileum(Kitazawa *et al.,*1997).
- Chicken motilin produce contraction in the smooth muscle of ileum and is blocked by atropine and tetradoxin(Liu and Burcher 2001).
- Galanin contracts the intestinal smooth muscle isolated from leghorn hens.Nifedipine inhibits the contractile response of the smooth muscle to galanin(Martin *et al.*,1993).
- Cisapride(5-HT_4-partial agonist) decreased effects of both 5-HT and 5-MOT(Pust *et al.*,1996).
- Acetylcholine produce contraction of the ileum by acting through muscarinic receptors(Chattopadhyay *et al.*,1992).
- Polyphloretin phosphate inhibited contraction to PGE_1,PGE_2,$PGF_{2\alpha}$(Chattopadhyay *et al.*,1992).
- 5-HT elicited ileal contraction and is decreased in presence of tetradoxin.
- Atropine, ketanserine, metthylsergide, methiothepine reduce the response to5-HT.
- 5-methoxy tryptamine(a mixed 5-HT_1, 5-HT_2 and 5-HT_4 agonist)produce contraction.
- Atropine at lower concerntrations selectively antagonize Ach. At higher doses inhibits histamine.
- Mepyramine specifically inhibited histamine (H_1) induced contraction.
- Methylsergide selectively antagonmized 5-HT induced contraction.
- Sodium meclofenamate at larger doses exhibited various degrees of non specific blockade of histamine,acetylcholine and 5-HT.
- Phenylbutazone antagonized Ach,histamine and 5-HT induced contractions.
- Diethyl carbamazine citrate at larger doses strongly antagonized histamine,Ach and 5-HT induced contractions.

2.0 MATERIALS AND METHODS

Preparation of physiological salt solution (PSS)

Compound	Tyrode
Nacl	8.0
Kcl	0.2
$CaCl_2$	0.2
$MgCl_2$	0.10
$MgSo_4$	-
$NaHCo_3$	1.0
NaH_2Po_4	0.05
KH_2Po_4	-
Glucose	1 or 2

All values are in g/l. Weighed accurate quantity of the ingredients and dissolved in one liter distilled water such that the physiological solution prepared should be clear, and if turbid it is advised to prepare fresh solution before the start of the experiment.

- Fresh entire gastrointestinal tract of healthy cock was obtained from a slaughter house in Puducherry.
- The caecum was lifted forwards and the ileocaecal junction was identified.
- A few centimeters of the ileal portion was cut and removed and immediately placed it in the watch glass containing physiological salt solution. The mesentery and adhering tissues were removed with gentle care. Utmost care was taken to avoid any damage to the gut muscle. The ileum was cut into small segments of 2-3 cm long.
- To one piece of ileum the thread was tied to top and bottom ends without closing the ileum, and mounted the tissue in the organ bath containing tyrode solution maintained at 32-35˚C and bubbled with air. The magnification from 5-7 folds and bath volume of about 25 ml was maintained, and the tissue was allowed to equilibrate for 30 min before adding Acetylcholine to the organ bath.
- The Acetylcholine(Ach) induces the contraction in the ileal smooth muscles which were recorded on Kymograph by using frontal writing lever. Contact time of 30 sec, and 5 min time cycle was kept for proper recording of the responses.
- The CRC was recorded till ceiling effect to Acetylcholine was obtained and the height of the response was measured.

- Various parameters were changed and responses were taken as magnification value 3 & 5, load/tension 0.5, 1.0 & 1.5 gm and tissue length 1.5, 2.0 cm.
- The experiment was repeated in presence of pioglitazone by irrigating tyrode solution with pioglitazone at varying doses of 4µg/ml and 8µg/ml.
- The concentration response curve of Ach in presence of pioglitazone at varied concentrations were recorded and labeled appropriately.
- The log doses and response height in mm were calculated and the concentration response curves of acetylcholine in the absence and presence of pioglitazone were plotted with log doses on X-axis and response height on Y-axis respectively.
- The effect of pioglitazone on CRC of Ach using chick ileum was observed based upon the shift of the curve in presence of pioglitazone whereas leftwards shift denotes the potentiation effect and rightwards shift denotes the inhibitory effect on Ach.

3.0 RESULTS AND DISCUSSION

Pioglitazone, brings about its insulin sensitizing effects in insulin target tissues like liver and adipose tissue through activation of the nuclear peroxisome proliferator activated receptor gamma (PPAR-γ) and thereby alleviate insulin resistance. The possible effect of Pioglitazone on pancreas to induce insulin release is yet to be proved in vitro or in vivo. In the present study the effect of pioglitazone on Acetylcholine induced smooth muscle contractions in chick ileum was studied to show the effect of pioglitazone on calcium release.

Pioglitazone was used at two different test doses (4µg/ml and 8µg/ml) and produced significant dose dependent improvement in Ach induced contractions in the concentration response curves.100 µg of Ach produced the response with a height of 96mm whereas in presence of pioglitazone, at 4µg/ml and 8µg/ml concentrations maximal response height of 115mm and 127mm was produced for respectively with the same concentrations of Ach.

Calcium plays a vital role in the coupling of stimulus recognition and insulin release by the pancreatic β-cells. (Malaisse, 1973).It was also reported that calcium is highly essential for the insulin release (Grodsky and Bennett, 1996; Milner and Hales, 1967). Some studies have also come out with promising results of sustained insulin release through increased intracellular Ca++ concentration using cationic ionophores (Wollheim et el., Ashby and Sparke 1995; Karl et al., Charles et al., 1975). The autonomic nervous system also participates in the regulation of rate of insulin secretion where cholinergic innervation enhances and adrenergic innervation diminishes the insulin secretion respectively. More over the specific stimulus for insulin release involves the entry of glucose into pancreatic beta cells via glucose transporter and its phosphorylation to glucose-6-phosphate. This triggers an intracellular calcium influx which in turn promotes the fusion of insulin containing secretary granules with the cell membrane and exocytosis of insulin (Craig and Stitzel, 2012). These data indicate that calcium acts as an essential trigger for insulin secretion from beta cells. In general sarcoplasmic reticulum plays an essential role in the regulation of calcium where depolarization of sarcolemma induces calcium release thereby leading to smooth muscle contraction (Otsu and Tada, 1993). The present study was designed to evaluate the potential the role of pioglitazone on Acetyl choline induced smooth muscle contraction which is a calcium mediated activity.

An *in vitro* study conducted on insulin release from chicken pancreas revealed the glucose and tolbutamide induced insulin secretion was inhibited by depletion of calcium in the medium while replacement of calcium resulted in insulin release (Naber and Hazelwood, 1977). In a recent study it has been shown that a novel thiazolidinedione - BLX-1002 which had no apparent affinity to peroxisome proliferator-activated receptors (PPAR) caused an increase in the high glucose induced insulin release from isolated mouse islets in vitro. This effect of BLX-1002 was mediated through augmentation of cytoplasmic calcium levels in a PI-3K dependent manner (Zhang et al 2009). A similar mechanism might be responsible for the observed potentiating effect of pioglitazone in the present study on Ach induced smooth muscle contraction in chick ileum in vitro.

CONCLUSION

Pioglitazone when used at two different test doses (4µg/ml and 8µg/ml) produced significant dose dependent enhancement in Acetyl choline (Ach) induced smooth muscle contractions in chick ileum. The height of concentration response curves obtained in the presence of

pioglitazone was greater than that obtained with Ach alone. This effect of pioglitazone may be attributed to its effect on augmentation of cytoplasmic calcium levels.

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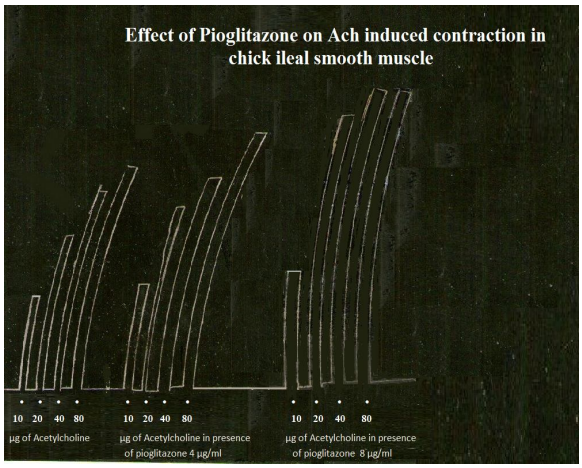

Fig. 1

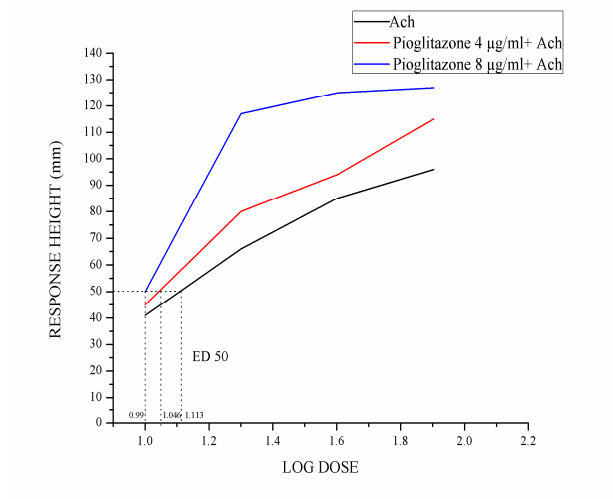

Fig. 2: Effect of Pioglitazone on CRC of Ach Induced Smooth Muscle Contraction in Chick Ileum

ED_{50} Value of Ach-12.97
ED_{50} Value in presence of 4µg/ml pioglitazone-11.12
ED_{50} Value in presence of 8µg/ml pioglitazone-9.77

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